Bacterial isolation and identification

Salmonella: Feces (1 g) are incubated in 10 mL of GN Haina (Difco Laboratories, Detroit, MI) for 18-24 h at 37° C, and Tetrathionate broth (Difco) for 40-48 h at 37° C. After the initial enrichments, aliquots (100 µl) are transferred to 10 mL of Rappaport-Vassiliadis R10 broth (Difco) which are incubated for 18-24 h at 37°C. Ten microliter aliquots of Rappaport-Vassiliadis R10 broth are then streaked onto Xylose-Lysine-Tergitol-4 (Difco) and BG Sulfa (Difco) agar for isolation of Salmonella. Plates are incubated for 18-24 h at 37° C. Isolated colonies characteristic of Salmonella are inoculated into triple sugar iron and lysine iron agar slants for biochemical confirmation. Presumptive positive isolates are serogrouped using serogroup specific antisera (Difco) and are sent to the National Veterinary Services Laboratory (Ames, IA) for serotyping. To deduce relationships among Salmonella isolates, DNA fragments resulting from digestion by restriction enzyme Xbal are separated by pulsed-field gel electrophoresis (PFGE) as described by Barrett et al. (1994). Image normalization and construction of similarity matrices are carried out using BioNumerics 2.5 (Applied Maths, Kortrijk, Belgium). Bands are assigned manually, and clustering is performed using the unweighted pair-group method with arithmetic averages (UPGMA) based on the Dice similarity index, utilizing an optimization parameter of 1.0% and a 0.5% band position tolerance.

Campylobacter: Fecal samples are diluted 1:9 (wt/vol) in sterile phosphate-buffered saline (PBS, 0.1 M, pH 7.2) and 100 μl aliquots are inoculated onto Campy-Cefex agar plates (Stern *et al.* 1992), and into Bolton Broth enrichment media (1 ml broth enrichments in Falcon 353047 tissue culture plates, 24 wells/ Becton Dickinson Labware, Franklin Lakes, NJ 07417). Agar plates and enrichment broth are incubated for 36-48 h at 42° C under microaerobic conditions (5% O₂, 10% CO₂ and 85% N₂). Presumptive *Campylobacter* colonies are selected by observation of cellular morphology and motility using a wet mount under phase-contrast microscopy. Isolates are identified by species using a commercial multiplex PCR (BAX[®] PCR, DuPont Qualicon, Wilmington, DE).

Enterococcus spp.: 100 μl aliquots of fecal dilutions (1:9 wt/vol, in PBS) are inoculated into 24 well tissue culture plates (Becton Dickinson Labware, Franklin Lakes, NJ 07417) containing1 ml of Enterococcosel broth (Becton Dickinson, Sparks MD 21152) per well. The enrichment broth is incubated for 18-24 h at 37° C, followed by streaking for isolation onto Enterococcosel agar (Becton Dickinson). Isolates are identified by species using a multiplex PCR (Jackson *et al.* 2004).

Generic *Escherichia coli*: 10 µl aliquots of fecal dilutions (1:9 wt/vol, in PBS) are streaked for isolation onto CHROMagar EEC[™] (Hardy Diagnostics, Santa Maria, CA) plates. The plates are incubated for 18-24 h at 42° C.